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Indicators of L-arginine metabolism and cardiovascular risk factors A cross-sectional study in healthy middle-aged men

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Summary. This study examines the relationship between traditional risk factors of coronary artery disease and indicators involved in the metabolism of L-arginine (plasma and urine L-arginine, plasma L-citrulline, serum creatinine and urine orotic acid). Our study population consisted of 40 healthy male volunteers aged between 35 and 55 years. We found an inverse association between serum creatinine and blood pressure, between plasma L-citrulline and blood pressure, as well as between urine L-arginine and blood pressure. We also found a positive association between plasma LDL-cholesterol and urine L-arginine and a negative correlation between plasma L-arginine and LDL-cholesterol. Orotic acid measured from urine was not associated with any of the indicators of L-arginine metabolism. Our results indicate that L-arginine metabolism is of profound significance for cardiovascular health. However, our study does not answer questions relating to causality. Further studies are needed to clarify the causal relationship between cardiovascular risk factors, especially elevated blood pressure and high LDL-cholesterol, and indicators of L-arginine metabolism.

Keywords: Amino acids – Atherosclerosis – L-Arginine – Creatinine – Citrulline

Introduction

Arterial endothelial cells can produce the vasoactive agent, nitric oxide (NO). They are responsible for the vasodilatation of arteries occurring during mental and physical stress (Palmer et al., 1987). Decreased basal production of nitric oxide is found in subjects with coronary disease (Quyyumi et al., 1997; Sumino et al., 1998). L-Arginine is the precursor of nitric oxide (NO) (Palmer et al., 1988). Nutritional supplementation with L-arginine can increase NO production in humans (Kharitonov et al., 1995) and possesses some effects typical of NO. L-Arginine can decrease blood pressure in saltsensitive

subjects (Campese et al., 1997). It also prevents the adhesion of monocytes to vessel walls in chronic smokers (Adams et al., 1997), attenuates aggregation of platelets in hypercholesterolemic humans (Wolf et al., 1997), enhances vasodilatation in smokers (Thorne et al., 1998; Campisi et al., 1999) and hypercholesterolemic humans (Clarkson et al., 1996) and reverses the aging-related decrease of vasodilatatory capacity (Chauhan et al., 1996). In humans, L-arginine has also been shown to reduce lipid peroxidation (Lubec et al., 1996) and to activate immunological defense systems (Park et al., 1991). In laboratory animals, L-arginine prevents atherosclerosis (Aji et al., 1997; Thorne et al., 1998), inhibits lesion formation after balloon angioplasty (Schwarzacher et al., 1997), decreases tobacco smoke-related infarct size (Zhu et al., 1996), attenuates cardiac hypertrophy (Matsuoka, 1996) and has beneficial cardiovascular effects in burn injuries (Horton et al., 1998). Due to its many effects some authors have proposed that L-arginine would be beneficial in routine clinical use (Cooke and Tsao, 1997; Chowienczyk and Ritter, 1997). However, only few attempts have been made to investigate the relationship between L-arginine metabolism and traditional cardiovascular risk factors. We performed this cross-sectional study in healthy middle-aged males. Our aim was to determine whether L-arginine and its metabolites show any correlations with traditional risk factors of heart attack. We chose four variables which are closely related with L-arginine metabolism: plasma L-arginine, serum creatinine, urine L-arginine and urine orotic acid. L-Arginine is produced from L-citrulline in the kidney (Rabier and Kamoun, 1995) and therefore L-citrulline is a possible limiting factor in L-arginine production. Orotic acid was taken as an indicator because L-arginine deficiency might lead to an increased secretion of orotic acid (Visek, 1986). Finally, creatinine is a probable indicator of the arginine pool of the body, since L-arginine is one of the precursors of creatine. Our results indicate that urinelevels of L-arginine, plasma L-citrulline and serum creatinine concentration are closely associated with blood pressure. Urine L-arginine and plasma L-arginine levels are also related with LDL-cholesterol.

Subjects and methods

Forty volunteers, middle-aged men (age range 35–52 years) were included in the study. None of them had any acute or chronic disease or were taking any medication. Four of the participants were current smokers. All subjects provided written consent for participation in the study. The study protocol was approved by the Ethics Committee of Kuopio University Hospital. Venous blood and urine samples were taken from the participants between 7 and 8 a.m. after ten hours of fasting. Amino acid levels of the blood and urine and orotic acid level of urine were analyzed in the Kuopio Regional Institute of Occupational Health and the other routine clinical chemistry analyses (total- and HDL-cholesterol, triglycerides, creatinine, renin, insulin, fibrinogen, urate and exact CRP) in the Kuopio University Hospital. LDL-cholesterol was calculated using Friedwald's equation. The blood pressure was measured from the right arm after 10 and 15 minutes of rest. The mean values of the measurements were used in the data analysis. The statistical analysis was carried out using the SAS system. Pearsons correlation value was used to evaluate the crude associations between variables and logistic regression analysis used to

Table 1. Description of the study population

Variable	Mean	Standard deviation	Range
Age (years)	44	4.5	35–52
Height (cm)	177	5.1	168–190
Weight (kg)	83	12.5	62–121
Plasma L-arginine (μmol/ml)	52	21.6	18–108
Urine L-arginine (μmol/mmol creatinine)	1.7	0.8	0.4–3.9
Plasma L-citrulline (μmol/ml)	26	8	10–44
Serum creatinine (μmol/l)	95	10	71–114
Urine orotic acid (μmol/mmol creatinine)			<0.4–3.9
Systolic blood pressure (mmHg)	123	11	104–155
Diastolic blood pressure (mmHg)	84	9	63–102
Total cholesterol (mmol/l)	6.0	1.0	3.7–9.1
Hdl-cholesterol (mmol/l)	1.4	0.3	0.8–2.7
Triglycerides (mmol/l)	1.5	0.8	0.7–4.3
Ldl-cholesterol (mmol/l)	4.0	0.8	2.3–6.0
Chol/Hdl-ratio	4.7	1.2	2.9–7.6
Plasma renin (μg/l/h)	1.8	1.4	0.2–6.1
Serum albumin (g/l)	45	3.3	36–51
Serum insulin (mU/l)	12	11	4–56
Plasma fibrinogen (g/l)	3.0	0.9	1.8–6.7
Serum urate (μmol/l)	340	60	229–472
Exact serum CRP (mg/l)	1.9	4.0	0–19

eliminate the confounding factors. Probability values less than 0.05 were considered as significant. The description of the study population is presented in Table 1.

Plasma assay for L-arginine and L-citrulline

The derivatization of L-arginine and L-citrulline with o-phthalaldehyde (OPA) was carried out according to Qureshi et al. (1984 and 1988) with slight modifications. For amino acid analysis (L-arginine and L-citrulline), the AminoQuant amino acid analysis system (Hewlett-Packard, Palo Alto, California, USA), HP 1090 Series II liquid chromatograph with a DR5 binary solvent delivery system, a HP 1046A fluorescence detector operating at 230 nm and 455 nm, an auto-injector and an auto-sampler are used. The automatic operation is under control of an HPLC ChemStation system. The separation of amino acids was performed on 5 μm HP ODS-Hypersil column (200 * 4.6 mm) obtained from HP. A precolumn (5 μm HP ODS Hypersil, 20 * 4 mm) was inserted between in analytical column and the injector. For channel A, a 0.03 N sodium acetate buffer was used containing 0.25 tetrahydrofuran, adjusted to pH 7.2. For channel B, a 0.1 M sodium acetate buffer-acetonitrile mixture (4:1) was used. Both buffers were filtered (Millipore, 0.45 μm filter type HA) before use. A mobile-phase gradient was used, starting with 96.7% A at 0 min, increasing B from 3.3 to 25% over 20 min and to 100% by the 21th to 23 min and returning to 96.7% A by the 24th. Each chromatographic experiment was completed within 25 min. The flow rate was 0.8 ml/min. The standard curve was found to be linear over the concentration range of 10 to 300 pmol/μl. The recovery was about 85% with coefficients of variation of 1.2%, 0.4% and 1.1% for L-arginine and L-citrulline (150 pmol/μl), respectively. Limit of detection was 10 pmol/μl.

Urine assay for L-arginine

Morning urine samples for L-arginine measurement were taken from the subjects. The urine samples were centrifuged at 2,500 rpm for 10 min and the supernatant was collected and stored at −20°C. Just before determination, the sample was thawed at room temperature. 0.1 ml of 100 μl/ml L-homoserine was added to each 1.9 ml urine sample as an internal standard. The test tubes were mixed and filtered. The determination of L-arginine in urine samples was based on precolumn derivatization with OPA and the analysis continued, similarly to the plasma assay. The standard curve was found to be linear over the concentration range of 10 to 300 pmol/μl. The recovery was about 98% and a coefficient of variation 2.4% (150 pmol/μl). The limit of detection was 10 pmol/μl.

Urine assay for orotic acid

Urine samples for orotic acid determination were the same samples as used in the L-arginine analysis. The urine samples were centrifuged at 2,500 rpm for 10 min and supernatants were collected and stored at −20°C. Just before determination, the sample was thawed at room temperature and filtered. The analysis system used in this study comprised HP 1090 Series II liquid chromatograph with an HP diode-array detector operating at 275 nm, an auto-injector and an auto-sampler. A strong anionxchange column (Whatman Partisil SAX 10 μm, 250 × 4.6 mm) was used. The measurement of orotic acid was carried out according to Brusilow and Hauser (1989) with slight modifications. The mobile phase, pumped at a flow-rate 1.2 ml/min, consisted of 0.8 M formic acid in 35% methanol adjusted to pH 2.8. The buffer was filtered (Miilipore, 0.45 μ filtertype HA) before use. The standard curve was found to be linear over the concentration range of 5 to 300 pmol/μl. The coefficient of variation was 2.4% (80 pmol/μl). The limit of detection was 5 pmol/μl.

Results

The results indicate a positive correlation between L-arginine and diastolic blood pressure and an inverse correlation between L-arginine and LDL-cholesterol (Table 2). Citrulline and creatinine correlate positively with each other ($r = 0.31$, $p = 0.03$) and inversely with diastolic and systolic blood pressure. Citrulline was positively correlated with fibrinogen, but no correlation between citrulline and creatinine with other specific risk factors was found (Table 2). The logistic regression analysis which included one of indicators of arginine metabolism (plasma L-arginine, urine L-arginine, L-citrulline or creatinine) with age, body mass index and total cholesterol/hdl-cholesterol-ratio revealed that diastolic blood pressure has a statistically significant ($P = 0.02$) relationship with serum creatinine and urine L-arginine ($P = 0.05$). According to regression analysis systolic blood pressure is statistically significantly ($p = 0.03$) associated with urine L-arginine. Orotic acid did not have any relationship with urine or plasma L-arginine or plasma L-citrulline. The mean plasma L-arginine concentration was 49 μmol/ml in those with measurable and 55 μmol/ml ($p = 0.4$ in those with a non-detectable amount of orotic acid in urine. The respective figures for citrulline were 26 μmol/ml and 25 μmol/ml ($p = 0.7$). The values of traditional risk factors of coronary disease in the two classes of orotic acid are presented in Table 3. Those with a measurable amount of orotic acid had lower triglyceride values than those

Table 2. Correlation (Pearsons correlation coefficients) between plasma and urinary L-arginine and its plasma derivative L-citrulline and serum creatinine with cardiovascular indicators (CVI)

CVI	Plasma L-arginine	Urine L-arginine	Plasma L-citrulline	Serum creatinine
Systolic blood pressure	0.11	−0.36*	−0.32*	−0.29*
Diastolic blood pressure	0.29*	−0.37*	−0.29*	−0.48**
Total serum cholesterol	−0.14	0.24	0.01	0.14
Serum hdl-cholesterol	0.11	0.28*	−0.09	−0.07
Serum triglycerides	−0.11	−0.12	0.01	0.09
Serum Ldl-cholesterol	−0.32*	0.35*	−0.06	0.18
Plasma renin	−0.08	0.01	−0.15	0.11
Serum albumin	−0.17	0.34*	−0.27*	0.18
Serum insulin	0.24	−0.10	0.06	−0.07
Plasma fibrinogen	−0.16	−0.15	−0.29*	0.01
Serum urate	0.09	−0.20	0.14	0.27
Exact serum CRP	0.09	−0.13	−0.26	0.02

* $p < 0.05$, ** $p < 0.01$.

Table 3. Cardiovascular indicators (CVI) and urinary excretion of orotic acid

CVI	Amount of orotic acid in urine (μmol/mmol creatinine)		p-value
	<0.4	≥0.4	
Systolic blood pressure	125	121	0.23
Diastolic blood pressure	84	84	0.95
Total serum cholesterol	6.0	6.0	0.96
Serum HDL-cholesterol	1.4	1.3	0.62
Serum triglycerides	1.8	1.2	0.01
Serum LDL-cholesterol	3.9	4.0	0.85
Plasma renin	1.6	2.0	0.44
Serum albumin	45	45	0.98
Serum insulin	14	9	0.06
Plasma fibrinogen	2.9	3.2	0.23
Serum urate	345	333	0.54
Exact serum CRP	0.7	3.4	0.06

with a non-detectable value of orotic acid. No other relationships between orotic acid and cardiovascular risk factors were found.

Discussion

The background of this study was to identify, whether there is any relationship between L-arginine metabolism and traditional risk factors of coronary disease. The most interesting finding concerns the relationship between serum creatinine and blood pressure. The inverse relationship between serum

creatinine and diastolic blood pressure is in line with an earlier finding. Aromaa et al. (1981) performed a follow-up study including more than 6,000 healthy males aged 40–59 years at the beginning of the follow-up period. Serum creatinine levels measured at the beginning of the study were found to be a significant risk factor of total mortality during the follow-up of 5 years. A creatinine value of less than 70 units indicated a twofold risk to die from any cause when compared with creatinine value of 80–89.

Creatinine can be used as a marker of the whole L-arginine pool reflecting arginine production by the kidneys and the arginine supplementation from the diet. From that point of view, a strong association between plasma L-arginine pool and serum creatinine level should exist. However, no statistically significant association was found between the levels of L-arginine and creatinine. On the other hand, a clear association between L-citrulline, the precursor of L-arginine, and creatinine was found. One possible explanation for this peculiar finding is that much of the biologically available L-arginine remains within the endothelial cells. Thus the plasma concentration of L-arginine poorly reflects the size of the entire L-arginine pool. On the other hand, citrulline is quite a good indicator of endogenous L-arginine, because a major part of the citrulline produced in the small intestine is transported to kidneys and transformed to L-arginine. Thus the negative correlation between blood pressure and L-citrulline reflects a profound correlation between endogenous L-arginine production and blood pressure. This conclusion is confirmed by the fact that there was the same kind of correlation between creatinine and blood pressure. Since both the precursor and derivative of L-arginine are inversely correlated with blood pressure, it is quite bizarre that L-arginine itself is positively correlated with blood pressure. One explanation could be poor loading of endothelial cells with L-arginine. Theoretically, an inverse relationship between the plasma level L-arginine and L-arginine content of endothelial cells is possible, since L-arginine shares a common transport mechanism with ornithine, lysine and cysteine. In particular, a high plasma level of lysine effectively prevents the transport of L-arginine into endothelial cells (Bogle et al., 1992). Unfortunately at present, there is no way of measuring the L-arginine content of endothelial cells.

The inverse relationship between LDL-cholesterol and plasma L-arginine is similar to that reported by Jeserich et al. (1992), who reported a lower plasma L-arginine value in individuals with high cholesterol levels when compared with people with normal cholesterol values. The inverse relationship between plasma L-arginine and serum cholesterol has not been confirmed by other authors (Oleesky et al., 1992). Thus clarification of the possible relationship between plasma L-arginine and serum cholesterol are needed.

The inverse relationship between urinary L-arginine levels with blood pressure and its positive correlation with serum LDL-cholesterol is somewhat confusing. In fact, one would expect that blood pressure and LDL-cholesterol levels both would have either positive or negative associations, if any, with urinary L-arginine. Urinary L-arginine secretion could be anticipated to reflect the renal capacity to produce L-arginine, but obviously the situation is not so straight forward. Probably also the transport mechanism of L-arginine

during tubular reabsorption might be involved. This is also a finding which has to be confirmed and evaluated by other workers.

Orotic acid levels seemed to be unrelated to other indicators of L-arginine metabolism. Thus we could not confirm the hypothesis that L-arginine deficiency is associated with orotic aciduria (Visek, 1986). It is possible that severe orotic aciduria occurs mainly in situations where there is a massive depletion of L-arginine, for example in severe infections.

In conclusion, our findings indicate that L-arginine plays a substantial role in cardiovascular health and is closely related at least with blood pressure and LDL-cholesterol.

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